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Current Landscape and New Paradigms of Proficiency Testing and External Quality Assessment for Molecular Genetics

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Abstract

Context—Participation in proficiency testing (PT) or external quality assessment (EQA) programs allows the assessment and comparison of test performance among different clinical laboratories and technologies. In addition to the approximately 2300 tests for individual genetic disorders, recent advances in technology have enabled the development of clinical tests which quickly and economically analyze the entire human genome. New PT/EQA approaches are needed to ensure the continued quality of these complex tests.

Objective—To review the availability and scope of PT/EQA for molecular genetic testing for inherited conditions in Europe, Australasia and the United States; to evaluate the successes and demonstrated value of available PT/EQA programs; and to examine the challenges to the provision of comprehensive PT/EQA posed by new laboratory practices and methodologies.

Data Sources—The available literature on this topic was reviewed and supplemented with personal experiences of several PT/EQA providers.

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Conclusions—PT/EQA schemes are available for common genetic disorders tested in many clinical laboratories, but are not available for most genetic tests offered by only one or a few laboratories. Provision of broad, method-based PT schemes, such as DNA sequencing, would allow assessment of a large number of tests for which formal PT is not currently available. Participation in PT/EQA improves the quality of testing by identifying inaccuracies that laboratories can trace to errors in the testing process. Areas of research and development to ensure that PT/EQA programs can meet the needs of new and evolving genetic tests and technologies are identified and discussed.

Introduction

Recent advances in genetic testing technologies and an increased understanding of the role of DNA variations in health and disease, have produced expansion of molecular diagnostics and led to an increased role for clinical genetic testing in patient management. Currently, molecular genetic, biochemical, and cytogenetic tests for approximately 2300 inherited genetic diseases are offered in clinical laboratories for disease diagnosis, carrier screening, prediction of clinical disease susceptibility, risk assessment, and prognostication of disease course.[1] Due to the rapid growth and the potential impact of genetic testing results on clinical management or reproductive decisions, quality management practices are essential at all stages of the testing process to ensure the accuracy and utility of these tests.

Quality management is defined as an ongoing effort that includes policies and procedures established and implemented for the purpose of providing accurate laboratory test results.[2] Quality management of the analytic component encompasses a variety of quality assurance processes designed to assure the performance of the test in the clinical laboratory.[3,4] This requires a system that includes both internal and external procedures that are described in national and international guidance and regulatory documents.[4–15] Internal quality assurance processes include measures to maintain analytic accuracy, such as quality control and personnel competency. External quality assessment measures include examination of laboratory procedures by a third party accreditation process and participation in proficiency testing (PT) or external quality assessment (EQA) programs. Most of the current quality assurance practices commonly used in genetic testing laboratories are designed for well-established technologies, such as targeted mutation analysis, that detect limited sequence variations in one or a small number of genes associated with a particular disorder or condition. Newer technologies, such as next generation DNA sequencing (NGS) and chromosomal microarrays, allow detection of a greater number of sequence variations or gene expression levels. These new tests exhibit a higher level of complexity owing to the sophistication of chemistry, hardware, and software innovations.[16,17] Therefore, it is unclear whether traditional methods of quality assurance and assessment will suffice for this higher level of complexity or whether new paradigms must be developed.

Proficiency testing is defined in ISO17043 as: “evaluation of participant performance against pre-established criteria by means of interlaboratory comparisons”.[18] This can be achieved through participation in a formal PT program. PT programs usually focus on the analytic results. External quality assessment also provides evaluation of laboratory performance on examination of external samples, but focuses more on the pre-analytic (pre-

examination) and post-analytic (post-examination) activities than PT. [18] In the absence of a formal PT or EQA program, laboratories can assess their performance through alternative assessment activities, such as exchanging samples with another laboratory performing similar tests, or by internal assessment.[19]

PT/EQA is an important component of clinical laboratory quality assurance. It provides a mechanism to compare analytical test performance among different laboratories, which is important for determining consistency of test results for a common analyte. Participants in formal PT/EQA programs periodically receive specimens (with a genotype unknown to them) which are tested in a manner similar to procedures used for their regular clinical samples. Participating laboratories return the results of the requisite analyses to the PT/EQA program, which then compiles the data and provides summarized results and educational insights to the participants. These programs provide an independent measure of laboratory performance in comparison with an external standard, or a mean value obtained by other participating laboratories. Participation in PT/EQA allows laboratories to recognize analytical and interpretive errors and may indicate internal problems with quality control, calibration, assay design or test interpretation. This is important because the majority of clinical molecular genetic tests are developed by individual laboratories and are not available as commercial test kits that are manufactured and evaluated in a consistent manner. Also, unlike molecular tests for infectious diseases, such as HIV and tumor markers which are typically used for patient monitoring and may be performed repeatedly, most molecular genetic tests for inherited disease are performed only once in a patient's lifetime. In this situation, errors may not be noticed through discrepancy with subsequent testing.

An international survey of molecular genetic testing laboratories determined that 74% of responding laboratories participate in PT/EQA which was suggested to correlate with higher quality assurance scores.[20,21] The most common reason cited by laboratories for failure to participate in PT was a lack of programs relevant to the clinical laboratory services offered.[21] In the U.S., formal molecular genetic PT programs are available from the College of American Pathologists (CAP)[22] for 27 tests for inherited diseases representing only a small fraction of the 1739 (US) or 2247 (worldwide) molecular genetic tests currently available.[1] However, the 27 CAP PT surveys are for tests with the greatest frequency of utilization, including factor V Leiden, prothrombin 20210A variant, cystic fibrosis, and fragile X syndrome. CAP and other PT/EQA providers also offer schemes for many other molecular genetic tests including, cytogenomic microarray analysis, pharmacogenetics, paternity testing, and HLA typing.

Another survey of clinical genetic laboratories in the United States indicated that increased participation in PT correlated directly with decreased PT failures and number of incorrect patient test reports, as reported by laboratories.[23] Published perspectives about the ability of PT to accurately measure routine laboratory performance, are conflicting, therefore this correlation may be difficult to prove.[24–30] Formal PT/EQA provides inter-laboratory comparison of specific samples and does not always examine the entire testing process, or day-to-day quality management issues that may impact laboratory performance. PT represents a "snapshot" and is not intended to provide a comprehensive evaluation of a

laboratory's quality assurance (QA) processes. Nonetheless, it has documented value for identifying problems that compromise the quality of laboratory test results.

Regulatory authorities and professional organizations recognize that PT/EQA is an essential component of quality assurance, and have developed policies and recommendations for inclusion of PT/EQA, where feasible, into laboratory practice. Several international organizations, such as the Clinical and Laboratory Standards Institute (CLSI) and the International Organization for Standardization (ISO) have published guidelines and standards related to proficiency testing for molecular diagnostic methods. The CLSI documents MM14-A, *Proficiency Testing (External Quality Assessment) for Molecular Methods*; GP27, *Using Proficiency Testing to Improve the Clinical Laboratory*; and GP29, *Assessment of Laboratory Tests When Proficiency Testing is Not Available*, offers guidance for the management and operation of PT/EQA for PT providers as well as molecular genetic diagnostic laboratories.[19,31,32] ISO/IEC 17043:2010, *Conformity assessment – General requirements for proficiency testing*, specifies the requirements for the competence of PT providers, and for the development and operational aspects of providing PT schemes. This guidance also describes methods of PT testing. [18] The ISO document, ISO15189, *Medical Laboratories-Particular Requirements for Quality and Competence*[3], recommends that laboratories participate in EQA and that these EQA schemes should provide clinically relevant challenges that mimic clinical samples and encompass all steps of the testing process, including pre- and post-analytical components.

In the U.S., the Secretary's Advisory Committee on Genetics, Health, and Society (SACGHS) was mandated by the Department of Health and Human Services (HHS) to report on the adequacy of genetic testing oversight and regulation and to identify gaps that could affect patient safety. In their 2008 report, *U.S. System of Oversight of Genetic Testing: a Response to the Charge of the Secretary of Health and Human Services*, SACGHS formulated recommendations to address the main gaps in genetic testing oversight.[33] One recommendation addressed the absence of formal PT programs for all genetic tests, particularly rare genetic disorders, and suggested that HHS promote the development of new PT products by investigating other performance assessment approaches, including method-based processes.

In this manuscript, we review PT/EQA as it applies to molecular genetic testing for inherited conditions in the U.S., Europe, and Australasia. We consider the successes and demonstrated value of available PT/EQA programs and examine the challenges posed by evolving laboratory practices and testing technologies. PT/EQA programs are often unable to assess the total laboratory testing process, focusing primarily on the analytic phase of testing (often neglecting the pre- and postanalytic phases), nor provide PT/EQA for all available tests. This critical evaluation suggests the need for research and development of targeted efforts to meet future PT/EQA needs.

PT/EQA-the current situation

Resources describing the scope of genetic testing

No formal mechanism exists either to capture information, such as disorders and genes tested or analytic methods about all genetic tests offered in the United States or worldwide, or to identify all laboratories that perform these tests. Data is limited to voluntary registries such as the GeneTests[34] and Orphanet.[35] These resources do not obtain information from laboratories that either decline to analyze samples submitted from outside of their own institutions or that decline to register for other reasons. Thus, the true scope of molecular genetic testing and the laboratories that perform such testing is unknown. The National Institutes of Health in the United States has recently developed another registry[36] to collect information about genetic tests offered in the United States, although it may not be more comprehensive than the registries that currently exist.

According to the GeneTests website, the number of disorders with available genetic testing worldwide has increased more than 20-fold between 1993 and 2012 (from 100 to 2300 tests).[34] Nucleic acid based testing is available for about 2000 of these genetic disorders; other diagnoses use biochemical, chromosomal, or other genetic tests.[1]

The Orphanet database, also a voluntary registry, has catalogued 5954 rare diseases, but only some of these have diagnostic testing available. The data base lists 5424 laboratories that offer testing (both research and clinical), mostly located in Europe.[35]

Molecular methods used for genetic testing

There are many molecular methods that can be used to detect mutations depending on the disorder and the associated molecular defects. For example, many common mutations cause cystic fibrosis; testing for this disorder is usually performed by targeted mutation analysis using a panel of the more frequent mutations (single or a few nucleotides) associated with severe disease phenotype. Duchenne muscular dystrophy is most often caused by deletions and duplications in the *DMD* gene, therefore testing for this disorder includes deletion/duplication analysis. Other commonly used molecular testing techniques include DNA sequence analysis, mutation scanning and methylation analysis. To estimate the percentage of tests using each method, we chose a random sample (~10%) of diseases with available molecular genetics diagnostic methods (2/26/09 Report of ~970 diseases obtained from GeneTests) and tabulated the method(s) used to detect mutations for each. Whole gene or targeted exon DNA sequence analysis was used by the laboratories to analyze 93% of the disorders in our sample (Table 1). Testing for 49% of the disorders in our random sample was performed using only DNA sequencing techniques. This fraction may increase as next generation DNA sequencing technologies transition to clinical laboratories. Testing for approximately 23% of the disorders in our sample utilized targeted mutation analysis and testing for 27% of the disorders utilized deletion/duplication analysis, usually as a follow-up to sequencing assays which may miss large deletions and duplications (Table 1).

Molecular genetic tests evaluated by formal PT programs

In the past two decades, formal PT programs have become an integrated part of clinical genetics laboratory practice. There are many regional, national and international PT programs. We describe here some of the larger programs that serve national and international participants, however, smaller programs that serve a more limited base or programs without publicly available information were not included. A comprehensive list of available molecular genetic PT/EQA programs can be found on the Eurogentest website.[14] In the United States, CAP is the largest provider of molecular genetic PT challenges.[22] In partnership with the American College of Medical Genetics (ACMG), CAP offers proficiency testing for 27 inherited genetic disorders, 5 pharmacogenetic loci and methods-based PT for cytogenomic microarray analysis, and for post analytical Sanger DNA sequence analysis to laboratories worldwide (Table 2). The samples distributed in the PT challenges are typically highly-purified nucleic acids extracted from human cell lines. This program provides participants with 3 samples per disorder twice per year. The methods-based challenge for Sanger DNA sequencing assays is currently focused on interpretation of electronic data files but will evolve to include a “wet” challenge utilizing extracted DNA and including DNA sequence analyses. The Centers for Disease Control and Prevention’s (CDC) Newborn Screening Quality Assurance Program (NSQAP)[37] offers PT for molecular cystic fibrosis screening. NSQAP requires laboratories to extract DNA from blood spotted on filter paper collection devices. The NSQAP sends five blood spots to participating laboratories on a quarterly basis which allows laboratories to perform all phases of the testing process, including DNA extraction from the appropriate matrix, within the PT context.

European PT/EQA challenges are provided by both national and international organizations (Table 2) with additional participation by laboratories from outside Europe. The European Molecular Genetics Quality Network (EMQN)[38], which is supported by user subscriptions, offers disease-specific EQA for a variety of genetic disorders as well as methods-based EQA for Sanger DNA sequencing, molecular cytogenomic analysis and mutation scanning. EMQN EQA programs provide 3 lyophilized DNA samples per disease to participants once each year. Participants are assessed on their ability to correctly genotype, interpret and report the results using their usual laboratory report format. The Cystic Fibrosis (CF) European Network[39] offers an external quality assessment scheme for cystic fibrosis molecular genetic testing to over 200 laboratories worldwide. This program “aims to evaluate the entire analytical process, from DNA sample receipt and genotyping up to the written report with the final interpretation of the data as it is normally being sent to the clinician who requested the genetic test”. The United Kingdom National External Quality Assessment Service (UKNEQAS)[40] provides external quality assessment for a range of inherited diseases currently tested in diagnostic molecular genetic testing laboratories by providing challenges using lyophilized DNA or dried blood spots. This program examines the analytical and post analytical stages, including evaluation of laboratory reports of testing and provides 3 samples per disorder per year to participants worldwide. The Italy-based Istituto Superiore di Sanità (ISS) addresses current practice, problems and future directions of interlaboratory comparisons. Their focus is PT/EQA for molecular genetic testing of a limited number of diseases. The PT materials utilized are DNA samples extracted from

lymphoblastoid cell lines. This program covers both the analytical as well the post analytical phases of testing and provides 6 samples per disease annually.[41] The Deutsche Vereinte Gesellschaft für Klinische Chemie und Laboratoriumsmedizin e.V. (DGKL)[42] in Germany offers a variety of test modules as well as methods based PT for DNA sequence analysis and DNA isolation for factor V Leiden genotyping. Lyophilized DNA is provided for all DGKL PT challenges, except the DNA isolation scheme for which whole blood samples are sent.

Since 2008 the Human Genetics Society of Australasia Molecular Genetics Quality Assurance Program (HGSA MGQAP)[43] offers 23 disease specific PT modules in collaboration with EMQN and the CF European Network. This program also offers 1 to 3 generic modules, per annum which are methods/techniques based, to enable a wider participation in the program. In particular, the generic modules are aimed at laboratories that do not participate in any of the disease specific modules, but use the same methods/ technology in their testing. One such module was an audit-based assessment of result reporting, for which laboratories were asked to provide de-identified copies of one positive, one negative and one not-tested report, received for analysis within a specific time frame. Another module was Mutation Detection and Biological Interpretation, for which laboratories were provided with amplified products and sequencing primers, and were required to genotype the fragment, detect the variation, and report a biological interpretation of the results. Laboratories were assessed for measures of the quality of data provided and result interpretation. Laboratories from Australia, Hong Kong, Indonesia and Saudi Arabia participate in this program, which has amalgamated with the RCPA QAP Pty/Ltd programs in 2010. There is also a PT program for cytogenetic testing offered through the HGSA.

These formal PT/EQA programs include some of the more common genetic disorders that are tested in many laboratories (Table 2). However, these tests represent only a small fraction of more than 2300 disorders with available genetic tests. PT/EQA providers select disorders, such as cystic fibrosis, that are tested by multiple laboratories to make the programs economically feasible, and usually perform customer surveys to assess needs prior to developing a new disease challenge. In 2003 EQA was available for 8.4% and 4.2% of available genetic tests in the United Kingdom and The Netherlands, respectively. However when these data are considered in the context of how frequently a particular test was performed, this represented 63% of the total number of cases reported in the United Kingdom and 48% of the cases reported in the Netherlands at that time.[44]

The availability of appropriate and diverse materials, such as blood from affected patients or characterized cell lines from which PT/EQA materials are derived also affects the availability of PT/EQA programs. It is not logistically or economically possible to provide a formal PT/EQA challenge for disorders that are tested in only 1 or a few laboratories or for those without a supply of available materials.

In the absence of formal PT/EQA schemes, laboratories in the US and elsewhere must evaluate the performance of their assays using alternative methods (alternative assessment). This can be accomplished by blinded retesting of previously tested samples, sample exchanges with laboratories performing similar tests, or by internal evaluation of data. Methods for alternative assessment are described in numerous guidance

documents.[3,19,31,45,46] PT/EQA providers such as CAP and UKNEQAS facilitate sample exchanges among laboratories for tests without formal PT/EQA schemes. The effect of alternative assessment on laboratory quality has not been evaluated.

Phases of testing and their evaluation by PT/EQA

To have the greatest value, PT/EQA challenges should evaluate performance in the pre-analytical, analytical, and post-analytical (pre examination, examination and post examination) stages of the testing process. Laboratories should process and analyze PT/EQA samples in the same manner as routine clinical patient specimens to the fullest extent possible. Successful PT/EQA participation should demonstrate proficiency in all examined phases of testing.

The pre-analytical testing phase includes the receiving, accessioning, labeling, and initial processing of the sample. When PT/EQA results are evaluated across laboratories, a common type of error is a sample switch or mislabeling that is probably caused by a clerical error in the pre-analytical phase. Even though the analytical process may be performed correctly, the clerical mistake made earlier in the testing process produces incorrect test results and reporting (Table 3). This type of error is of particular concern because laboratory workers are frequently aware that the sample was provided for PT/EQA purposes.[47] However, because PT samples do not enter the laboratory workflow in the same way that routine patient samples do, there may be hidden, uncontrollable factors in the acquisition of PT specimens that could make such errors more likely.

The next step in the testing process is nucleic acid isolation, which can be performed using laboratory developed methods or with a variety of commercially available reagent kits and instruments. The isolated DNA or RNA obtained through these extractions may be derived from blood, bone marrow, formalin-fixed paraffin-embedded tissue, or fresh tissues. The quality of the isolated nucleic acids is central to the success of molecular genetic testing methods.

The ideal PT/EQA sample would be a clinical specimen, because it would most closely represent what is actually tested in a clinical laboratory and would permit the evaluation of all phases of the testing process, including the DNA extraction step.[47] It is, however, often difficult or impossible for formal PT programs to obtain sufficient quantities of appropriate, high-quality, safe, homogeneous and stable clinical samples to supply all of the laboratories participating in the PT event with the exact same specimen material.[44,47] For many genetic disorders, it is difficult to recruit a sufficient number of patients to reflect the variety of mutations that would be required for a comprehensive PT/EQA program, especially for very rare diseases or mutations. Institutional Review Board (IRB) and informed consent requirements may also present an obstacle to the use of clinical specimens. Clinical samples are often incompletely characterized, and may yield unexpected genotypes, including patterns of mosaicism, minor clonal populations[48] or previously unidentified alleles.[49,50] It is also difficult to transport potentially infectious whole blood across international borders.[51] DNA extracted from cell lines, which is available in almost unlimited supply, is often substituted as PT/EQA samples. The disadvantage of this practice is that the nucleic acid isolation phase is not performed by the participating laboratory and cannot be evaluated as

part of the proficiency testing process. In addition, sources of potential PT materials are limited in the range of diseases and mutations represented. For these reasons, most PT/EQA samples are not directly comparable to the samples usually received and cannot be processed in the same way as actual clinical samples.[47] Anecdotal evidence also indicates that some analytic techniques such as next generation sequencing and cytogenomic microarrays often do not perform optimally with DNA isolated by some methods used by external sources.

The analytical phase encompasses the actual testing of the analyte. Each participating laboratory performs the testing using its own validated method(s). In surveys with many subscribers, such as cystic fibrosis, it is not uncommon for participants to utilize a wide variety of commercially available assays as well as laboratory developed tests. Although PT/EQA performance is usually excellent for molecular genetic tests,[52,53] analytical errors do occur. Some errors may be associated with the design of the assay. For example, previously unrecognized polymorphic variants located in amplification primer binding sites may preclude effective amplification and detection of the actual mutation. One PT/EQA scheme for cystic fibrosis determined that a particular laboratory developed test could not accurately detect the 621 +1G>T mutation in the PT sample. The laboratory subsequently removed this mutation from its clinical assay.[54] In a similar case, a method-specific artifact produced a false result in a hereditary hemochromatosis PT challenge.[55] Poor performance on a PT/EQA challenge alerted another laboratory that the primer binding site in a BRCA assay was too close to the target mutation nucleotide which hindered the detection of the variant.[44] PT/EQA testing is one mechanism to alert laboratories to such problems and to indicate changes to avoid future errors. The results of PT/EQA can also compare laboratory performance with different assay methods among laboratories.[54] This has been especially useful when assessing the accuracy of trinucleotide repeat sizing. For example, it is very important to accurately size fragile X premutation expansions to correctly predict the risk of allelic expansion, premature ovarian failure and fragile X-associated tremor/ataxia syndrome. Inaccurate sizing, by even a few triplet repeats, could affect prenatal diagnosis and risk of expansion estimates or conflicting reports on different family members with similar repeat sizes tested in different laboratories. If laboratories cannot accurately size the fragile X triplet repeat, in comparison to results of the other PT/EQA survey participants or to the previously measured repeat size of the sample, additional calibration of the assay is warranted. Poor performance by a number of laboratories in the 2002 and 2003 UKNEQAS fragile X PT/EQA schemes resulted in the development of consensus testing and reporting guidelines in the United Kingdom.[44] The American College of Medical Genetics assesses results from a subset of the CAP proficiency testing surveys; if a particular problem occurs at a high frequency, disease specific testing practice guidelines are established and published.[47] ACMG guidelines for fragile X testing[56] were written in response to suboptimal performance on the CAP fragile X proficiency survey.

During the post-analytical phase of testing, the test results are reviewed and interpreted. Most of the molecular genetic PT modules offered by the CAP have an analytic as well as a clinical interpretation component, which are graded separately.[44] Participants provide an interpretation of the detected genotype within the context of a described clinical scenario (presentation of the patient), such as whether the identified mutations were consistent with the diagnosis of the disorder, or whether the genotype indicated a genetic carrier. However,

other components of the laboratory report, such as compliance with existing guidance or recommendations[15] describing required information elements, accuracy about the indication(s) for testing, test performed, results obtained, and appropriateness of follow-up guidance, are not evaluated. In European EQA programs, such as UKNEQAS, CF European Network and EMQN, the participants are required to submit results in their usual clinical reporting format. These reports are assessed for accuracy of genotyping, the appropriateness of the interpretation and clerical accuracy.[38–40,44,51,57] This allows evaluation of the laboratory's interpretation of the analytic results in the context of the mock clinical data supplied with the DNA samples and permits assessment of other important elements in the laboratory report, such as residual risk calculations, recommendations for further testing, use of proper genetic nomenclature and accurate inclusion of patient identification such as name, gender and birth date. Correct interpretation of the analytical result is essential because patient management will, in many cases, be based on a combination of the analytic result and the final interpretation. This is especially important for genetic testing where the test may only be performed once in a patient's lifetime. Because a genotype, per se, is not informative, proper result interpretation requires integration with other information that may include family history, ancestry, and knowledge of genotype/phenotype associations, which is important in clinical genetics because insights about the causes of disease and the effects of various mutations or combinations changes rapidly, and may affect clinical management of the patient.

Another important component of the post analytic analysis is laboratory reporting of the identified mutation using the appropriate gene mutation nomenclature, which makes clear to the physician who directly interacts with the patient which sequence change is identified. This may also include a reference to "common" nomenclature, such as "factor V Leiden" which is not consensus nomenclature but may be helpful in the report because it is most familiar to clinicians.[44,51] Use of incorrect or ambiguous nomenclature can lead to errors in interpretation, treatment selection, and testing other family members or their result interpretations, especially if testing is performed in different laboratories.

Grading

Methods of grading proficiency test results vary with the provider, the specific scheme and the analyte. Some providers, such as CAP, grade survey results on the accuracy of the genotypic result and the interpretation. For example, CAP has recently started to grade sizing of Huntington disease and myotonic dystrophy repeats in all size categories but has graded interpretation at 80% consensus for many years. Many of the CAP surveys are graded based on consensus of 80% of participants; if this level of consensus is not reached, the challenge remains ungraded and "educational". Other providers, such as EMQN, provide numerical scores based on genotyping, interpretation and reporting.[38] Each EMQN participant receives an individualized report with their scores and comments from the evaluators including areas of their test report that need improvement. Scoring of EMQN schemes depends on the analyte and the scheme. For example, there are 2 EQA schemes for *BRCA* gene testing (breast cancer risk). The *BRCA* Full scheme assesses genotyping, biological and clinical interpretation whereas the EMQN *BRCA* Geno scheme assesses only genotyping and biological interpretation. Other EMQN schemes, such as congenital adrenal

hyperplasia (CAH) and hereditary deafness (*GJB2/GJB6* mutations), score genotyping only. These differences reflect the reporting practices of the participating laboratories. Finally, it is often challenging for proficiency testing programs to compare results due to the use of different methods, different calibration standards and different cut-off values by participants. These issues do not usually affect molecular genetic testing for inherited genetic disease because most results are qualitative, but they are important for tests such as *BCR/ABL1* or viral load, where the results are quantitative. PT/EQA programs cannot effectively grade some quantitative surveys due to the lack of calibration materials, standardized values for normal ranges, and cut-off values.

The role of Laboratory Proficiency Testing in Quality Management

Participation in PT/EQA allows laboratories to compare their performance against the range of responses provided by a group of peer laboratories. These comparisons are most effectively used to influence laboratory practice when they are systematically integrated into a Quality Management System (QMS) (defined by the International Organization for Standardization as: “a management system to direct and control an organization with regard to quality”).[3] As part of QMS, a laboratory must participate in relevant PT/EQA schemes and ensure that the cost is integrated into the budget with sufficient time and staff resources assigned to support participation. In addition, the laboratory must ensure that the results of PT/EQA are properly considered, disseminated and implemented to improve laboratory testing. Participation in appropriate PT/EQA and/or alternative assessment is a requirement within the CAP, ISO, and Australian accreditation processes.

The Quality Policy implemented through a QMS defines the overall approach to PT/EQA and how PT/EQA challenges are appropriately handled in the laboratory. The QMS should include clearly defined laboratory procedures for receipt of performance results from a PT/EQA agency or other assessment protocols, such as alternative assessment. This procedure will normally follow three phases. First, the laboratory director is required to disseminate the data to staff without delay. This conveys to staff the importance of participating in PT/EQA schemes and enhances the educational content of the exercise. The performance data must be reviewed for any indication of deficient performance that requires immediate corrective action to avoid errors. PT/EQA data should be routinely discussed in the most appropriate meetings of management, quality team and staff so that lessons learned can be discussed and, if necessary, changes in standard operating procedures can be quickly integrated into the QMS. PT/EQA data provides an opportunity for a laboratory to compare its performance with peers as well as celebrate and praise the staff when performance is exemplary. Second, PT performance data should be presented during management review meetings to examine any recurrent deficiencies which require correction or improvement. Finally, PT/EQA records should be stored and formatted for external audit by an accrediting agency.

Laboratories may experience a lapse or error in PT/EQA performance. Single occurrences of poor performance should be logged as an incident and used as an opportunity to review procedures and make improvements. A careful evaluation of the error may determine whether there is a system failure that may require re-design of a test, more frequent

instrument calibration, or adjustments to training procedures. However, PT/EQA may detect serial or persistent failures which the laboratory is obligated to address with a more fundamental review. Some PT/EQA providers report that laboratories have discontinued testing services following instances of poor performance in PT/EQA.[44] This indicates the importance that laboratory directors assign to this external comparison and emphasizes the role of PT/EQA as an educational tool and mechanism to improve performance in clinical laboratories. In some countries, PT/EQA providers are required to report poor and recurrent poor performance to an official monitoring agency. This agency may have a role for ensuring that corrective actions are designed to address poor PT/EQA performance. Ultimately they may have the power to escalate their intervention to involve the host institution of the laboratory concerned as well as regulatory agencies.

Evidence of the value of PT/EQA in ensuring good laboratory performance

Although few studies have addressed whether participation in PT/EQA programs directly improved laboratory performance, there is empirical evidence showing that the educational aspect of PT/EQA does help laboratories detect errors in their testing protocols and identify problems associated with their assays.[2] One study reviewed three rounds of PT data from 2002 (any provider) from approximately 6300 CAP accredited laboratories.[58] The study evaluated whether laboratories corrected deficiencies identified by PT, or whether they continued to have unsuccessful PT performance. The analysis indicated that about 90% of the PT problems were resolved after the first round of PT and 99% by the third round, suggesting that the laboratories had successfully corrected mistakes identified by PT performance. Other studies have shown that participation in the CAP Calibration Verification/Linearity Survey, which examines calibration verification and analytical measurement range of a number of non-genetic analytes (chemistry, immunology, hematology, etc) is associated with fewer PT failures.[59,60] A UK NEQAS PT/EQA scheme for hemophilia A from 2003 failed 4 laboratories based on poor performance in the evaluation of their clinical report. In subsequent surveys, only 1 laboratory failed to report properly, suggesting that the educational value and improvement of testing practices were derived from this exercise.[51] Another value of PT/EQA is the opportunity for laboratories to analyze unusual samples, not often encountered in their service, eg mosaicism of variants in fragile X syndrome.

The UKNEQAS PT/EQA program emphasizes interpretation of data and its implications for both the patient and the family. One summary of data indicates that poor PT/EQA performance due to interpretation errors has decreased between 1997–2006.[44] This may be derived from continued participation in PT/EQA.

Analysis of PT/EQA results has also revealed inconsistent results stemming from the lack of uniformity in practices among laboratories. Once these issues were identified, steps were implemented to harmonize practices among the laboratories. Poor and inconsistent performance of laboratories participating in a PT/EQA scheme for fragile X syndrome led to consensus testing and reporting guidelines development in the United Kingdom[44] and the United States.[56] Due to ambiguities in the ways laboratories report sequence variations, PT schemes (such as UKNEQAS) strongly suggested that genotypic PT/EQA results should be

reported using the Human Genome Variation Society (HGVS) standard nomenclature.[44,51] Participants in the CAP's disease-specific surveys typically report results using the common allele names; however, CAP requires the use of the HGVS nomenclature for participants in the Interpretation of Sequence Variants in Rare Disorders PT scheme and is moving towards the integration of the consensus nomenclature for all schemes.

Limitations of PT

To assess a given test, PT/EQA should evaluate the capability of a particular assay to identify a range of possible test results, or alleles, either during an individual challenge or over the course of several challenges. Considering this, proficiency testing for a given disease or genetic variant may be limited by:

1. Issues related to the limited availability of resources including appropriate samples and reference materials,
2. Logistical and practical difficulties encountered including the inability to offer PT/EQA for all genetic disorders, especially those tests for rare disorders or performed by a small number of laboratories
3. Limitations in the PT process for evaluation of laboratory performance.

Ideally, PT/EQA should evaluate the ability of the laboratory to identify all genotypes of interest for a particular disorder, which relies on the availability of appropriate samples. For simple tests such as Factor V Leiden or prothrombin 20210A, very few alleles are tested in a clinical setting, thus relatively few PT/EQA samples are required to represent the population variant affecting patients. For other disorders such as cystic fibrosis, there is a large variation in the number and composition of alleles included in clinical assays. Some assays only examine the 23 alleles recommended for carrier screening by the American College of Medical Genetics and the American College of Obstetricians and Gynecologists (ACMG/ACOG)[61], while other laboratories offer assays that test over 100 alleles, some of which are unique to particular patient populations. Many of the alleles included in the more comprehensive CF assays are very rare, and it is often difficult to obtain samples from patients or cell lines for PT/EQA. Another difficulty is the lack of highly characterized reference materials or calibrators with which to evaluate potential PT/EQA materials. This is especially important for DNA fragment sizing assays, such as triplet repeat testing, but may also be a factor for qualitative assays.

Proficiency testing programs also encounter logistical and practical problems. Most of the 2300 clinical genetic tests are offered in only one or a few laboratories, making provision of a formal proficiency survey logistically and economically difficult. Many multiplex genetic tests may simultaneously detect dozens of alleles (eg CF or pharmacogenetic loci). It is logistically impossible for PT/EQA programs distributing 3 samples 1 or 2 times a year to provide a sufficient number of different samples to adequately challenge the variety of genotypes represented in all patients or all assays for a particular genetic disorder. DNA sequence analysis identifies mutations in any part of a gene and can pose a unique challenge in interpretation. Lastly, PT/EQA typically targets well-characterized conditions and mutations. However, new disease associations are discovered regularly and genotype-

phenotype correlations are often not fully elucidated until years after the initial discovery. Allelic heterogeneity and the clinical impact of some sequence variants, such as missense mutations and complex variants, pose considerable challenges for interpretation when functional studies have not yet been performed or were inconclusive. An example of this situation is pharmacogenetic testing for *CYP2D6* variants. Many tests for this gene include polymorphisms with undefined effects on CYP2D6 enzyme activity. These less well characterized variants are challenging for development of reference materials, reporting, and interpretation of PT/EQA results. Formal PT/EQA might become available for a greater number of inherited conditions in the future, but it will probably not be feasible for such programs to cover all conditions and available tests.

Approaches to address limitations of proficiency testing: Methods-based PT/EQA

Proficiency testing which is designed to evaluate performance of analytic methods rather than specific genotypic assays has been proposed as a complementary mechanism to more broadly assess laboratory performance.[45,62–64] Methods-based PT/EQA examines technologies common to many genetic tests such as DNA purification, PCR amplification or DNA sequencing, and allows evaluation of critical analytic steps for individual assays that are not directly assessed by traditional PT/EQA schemes. It also permits inter-laboratory comparisons and can highlight analytical practices, such as DNA quantification that could be optimized for improved performance.[62]

The European Commission funded a project (EQUAL) [Full program title: “Multinational external quality assay (EQA) programs in clinical molecular diagnostics based on performance and interpretation of PCR assay methods including dissemination and training”] to develop and evaluate the utility of methods-based EQA to address methodological procedures and analytical proficiency in molecular diagnostic test performance independent of the target. Three EQUAL pilot projects for qualitative analysis, quantitative PCR, and DNA sequencing (EQUAL-Qual, EQUAL-Quant and EQUAL-Seq) were initiated.[62,65,66] The results from these pilot studies identified many areas of laboratory performance that varied considerably between participants (Table 4) and suggested areas that could be targeted for improvement.

As a result of relatively poor laboratory performance in the EQUAL-Seq project, specific training highlighting analytic and methodological skills were subsequently offered to the participating laboratories. A significant improvement of technical and interpretative skills was demonstrated in a confirmatory second round of EQA.[67]

Currently, methods-based proficiency testing is available for a variety of assays (Table 4). EMQN offers a methods-based scheme for Sanger sequencing. Participants receive amplicons to characterize, identify and report the sequence variants using the proper nomenclature. They also provide their raw sequence data which is evaluated by EMQN for quality scores, quality read length and quality read overlap.[63] In the United States, CAP has launched an electronic DNA sequence analysis survey. In 2012, this survey will send a set of primers and three DNA specimens. Participants will identify all variants and report them using accepted nomenclature standards. In the future, both CAP and EMQN plan to offer methods-based surveys to address the performance of NGS.

Synthetic samples

Synthetic DNA samples can be used for PT/EQA. These samples may be composed of plasmid DNA containing specific sequences or PCR amplicons. The CF Network tested the usefulness of a synthetic reference material containing 6 homozygous mutations in the cystic fibrosis gene (CFTR) and one polymorphism for suitability as proficiency testing material.[68] A majority of the laboratories in the study successfully identified the mutations, although some technical difficulties, such as incorrect genotyping (10/197 participants), or absent, incorrect, or insufficient interpretation (33/197 participants) regardless of genotype were reported. This synthetic sample was supplied in a blood-like matrix which also permitted evaluation of the DNA extraction step. In the U.S., artificially constructed CF mutation samples designed to mimic extracted human genomic DNA produced similar results.[69]

Discussion

Proficiency testing has demonstrated value as an important laboratory quality assurance tool, and has helped laboratories identify issues related to test design and performance. In addition, the ability to compare laboratory performance with others using the same or different methods on identical samples can highlight issues related to test methodology or interpretation or may inform development of best practice guidelines and standard policy.

Adoption of new and complex testing technologies, such as next generation sequencing assays, will require modifications to PT/EQA design and provision. In contrast to traditional genetic tests, which identify only a few mutations or perhaps the whole sequence of a well-characterized gene, next generation sequencing has the capacity to examine the sequence of large gene panels, the exome or the entire human genome, with an almost infinite variety of possible variants. Proficiency testing programs for next generation sequence assays need to monitor the ability of the laboratory to detect mutations in any part of the genome included in their validated test. In addition to the analytic phase of the testing process, the data analysis and interpretation of NGS is considerably more complicated than sequencing tests with smaller scope. PT/EQA can be used to compare performance among laboratories during all phases of NGS testing and may provide important indicators of which steps of the testing process are problematic. This information may not be readily discernible through daily quality control practices, and PT/EQA could be an important tool to assess whether the testing algorithm is sufficient to detect a loss of sensitivity or specificity for the detection of sequence variations that may only be evident when comparing results among laboratories. Interpretation of the analytic test result of such large scale analyses is difficult and uncertain because the effect of individual mutations, the function of each gene and its interaction with other genes in the genome has not yet been determined. PT/EQA schemes to assess the ability of the laboratory to interpret and report complex data could be quite informative. It is clear that novel and innovative PT/EQA challenges will need to be developed to assure the quality of these new tests.

Proficiency Testing or EQA should include a sufficient number of analytes to provide a reasonable estimate of interlaboratory comparability. For example, proficiency testing for factor V Leiden testing assesses the laboratory’s capacity to identify one of three possible genotypes per sample (normal, factor V Leiden heterozygote, and factor V Leiden

homozygote). Developing a PT program to gauge inter-laboratory comparability in this example is fairly straight forward. Proficiency testing for disorders with many disease associated alleles, such as cystic fibrosis or a next generation sequencing test presents additional challenges. Current proficiency testing can only assess a subset of possible mutations per challenge. Is the current process sufficient for achieving a credible interlaboratory comparison for very complex tests? The limited availability of characterized DNA materials and the cost associated with increasing the number of PT samples can be significant barriers for a PT/EQA program with a goal to offer comprehensive challenges.

Significant research, needs assessment, and pilot testing should be performed to ensure that proficiency testing addresses the changing needs of genetic testing technology, an evolving knowledge base, and continues to be a relevant and useful quality assurance tool. Some of the research topics may include: ways to provide PT/EQA for the increasing number of new tests, many of which are offered in only one or a few laboratories; including all phases of the testing process, rather than just the analytical phase; develop novel approaches for effective multiplex genotype testing challenges; and PT/EQA strategies to assess new technologies, such as next generation sequencing. Additional research will define PT/EQA improvements to promote the quality of laboratory testing, interpretation, and reporting.

The majority of genetic tests are done in only one or a few laboratories. Many of these tests use the same technology, such as Sanger or next generation sequencing to examine a particular gene or set of genes. Many other tests share similar methodology, such as DNA/RNA purification, PCR amplification, or MLPA. Other aspects such as result reporting are also common across tests. Research to develop and evaluate novel method or technology based PT/EQA schemes may simultaneously assess the performance of many tests. Schemes that evaluate the quality of laboratory reports for example, the CF Network, UKNEQAS and EMQN could also be developed more broadly. In addition, research should be conducted to assess the effectiveness and relationship of the commonly used methods of alternative assessment, such as sample exchange or blinded retesting of previously tested specimens, to the accuracy of routine laboratory test results. Information gleaned from these studies may provide guidance to laboratories for effective methods of alternative assessment.

Much work needs to be done to ensure that PT/EQA programs can meet the needs of new and evolving genetic tests and technologies. An important first step would be to design a scientific approach to collect data on the impact of PT/EQA on laboratory testing quality. Such studies could measure the relationship between PT performance and the accuracy of routine test results in the same laboratory, and also could be expanded to include PT/EQA for the newer testing technologies, including microarrays and next generation sequencing. Information from these studies may inform the development of improved proficiency testing programs, professional guidelines, and regulations.

A variety of research and development projects will address some of the current limitations of PT/EQA, including the shortage of characterized reference materials, both naturally occurring and synthetic, that can be used as PT/EQA samples. Cell lines can be created from patients with genetic disorders that are currently part of PT/EQA programs and also

disorders for which PT/EQA would be useful, but for which programs do not yet exist. Consideration should be given to each disorder to ensure that a comprehensive set of reference materials, containing as many clinically significant alleles as possible, will be developed. In addition, development of synthetic reference materials containing many alleles for a given disorder simultaneously should also be considered. These materials must be evaluated by testing in a number of clinical laboratories using a variety of assays and technologies before they are used as PT/EQA samples.

Proficiency testing has been, and should remain, an integral part of laboratory quality assurance. In the next few years, we hope to conduct and facilitate these and other research projects to evaluate and improve the quality of proficiency testing for molecular genetic testing.

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Table 1

Methods Used to Test for Inherited Disorders

Method Used	% diseases tested
Sequencing	93
Transcription-Mediated Amplification	23
Deletion/duplication analysis	27
Mutation scanning	18
Methylation analysis	2

Analysis of a random sample (~10%) of diseases for which molecular genetic testing methods are used (2/26/09 Report of ~970 diseases obtained from GeneTests), determined that testing for ~93% of these diseases utilized DNA sequencing methods in at least some of the laboratories that offered testing. Forty nine percent of the diseases in the sample were tested using only sequencing techniques.

Table 2

Proficiency Testing/External Quality Assessment (PT/EQA) Provider Schemes for Inherited Disorders

PT/EQA Provider	Analytes Covered	Molecular Genetic Challenges Per Year	Sample Types	Phases of Testing Covered
College of American Pathologists (CAP)[22]	*Disease-specific schemes*: factor V Leiden, fragile X syndrome, hemachromatosis, MTHFR, Prader-Willi/Angelman syndrome, prothrombin, cystic fibrosis, Huntington disease, Friedreich ataxia, hemoglobin S/C, Duchenne muscular dystrophy, myotonic dystrophy, RhD, spinal muscular atrophy, spinocerebellar ataxia, BRCA1&2, Connexin-26, multiple endocrine neoplasia type 2, Canavan disease, familial dysautonomia, Tay-Sachs disease, mucolipidosis IV, Bloom syndrome, Fanconi anemia, Gaucher disease, glycogen storage disease type 1A, Niemann-Pick disease type A. Pharmacogenetic markers. *Methods-based schemes:* DNA sequencing, microarray genomic copy number assay	*Disease-specific schemes*: 3 DNA samples 2X/yr; *cystic fibrosis*: 2 samples 2X/yr; *Pharmacogenetic markers*: 2 DNA samples 2X/yr, *Post-analytical DNA sequencing scheme*: 3 electronic challenges 2X/yr; *Microarray genomic copy number assay*: 2 DNA samples + 1 paper challenge 2X/yr	*Disease-specific, pharmacogenetic and microarray genomic copy number assay schemes:* extracted DNA; *Post-analytical DNA sequencing scheme:* One CD-ROM containing DNA sequence electropherogram files	*Disease-specific, pharmacogenetic and microarray genomic copy number assay schemes:* genotyping and interpretation; *Post-analytical DNA sequencing scheme:* interpretation
European Molecular Genetics Quality Network (EMQN)[38]	*Disease-specific schemes:* Y-chromosome microdeletions, BRCA1&2, CAH, Charcot-Marie-Tooth disease, Familial Adenomatous Polyposis Colon Cancer, GJB2 and GJB6, HNPCC, Monogenic Diabetes, Marfan syndrome, Porphyria, Hereditary Recurrent Fevers, myotonic dystrophy, Duchenne muscular dystrophy, fragile X syndrome, Friedreich ataxia, Huntington disease, haemochromatosis, multiple endocrine neoplasia type 2A, PKU, Prader-Willi/ Angelman syndromes, retinoblastoma, short stature homeobox gene testing, spinocerebellar ataxia, spinal muscular atrophy, Von Hippel Lindau, Wilson disease. *Methods-based schemes:* Sanger DNA sequencing, arrayCGH, Next Gen sequencing (pilot)	*3 DNA samples* + mock clinical data 1X /yr	Lyophilized DNA	Genotyping, interpreting and reporting.
United Kingdom National External Quality Assessment Service (UK NEQAS)[40]	*Molecular genetics*: Angelman syndrome, Becker/Duchenne muscular dystrophy, BRCA1&2, cystic fibrosis, factor V Leiden, familial adenomatous polyposis, fragile X syndrome,	Most disease-Specific schemes: 3 samples 1X/yr. Molecular testing on blood spots: 3 samples 4X/yr	Lyophilized DNA, Whole blood spotted on filter paper and dried	Genotyping, interpreting and reporting.

PT/EQA Provider	Analytes Covered	Molecular Genetic Challenges Per Year	Sample Types	Phases of Testing Covered
	hereditary and motor sensory neuropathy, Huntington disease, HNPCC, hereditary neuropathy with liability to pressure palsies, MCADD, mitochondrial disorders, molecular rapid aneuploidy testing, myotonic dystrophy, Prader-Willi syndrome, spinal muscular atrophy, spinocerebellar ataxia. *Molecular testing on dried blood spots*: cystic fibrosis, MCADD. *Microarray CGH*			
Human Genetics Society of Australasia (HGSA)[43]	23 disease specific PT modules in collaboration with EMQN and the CF Network. Y chromosome deletions (AZF), familial breast cancer (BRCA), congenital adrenal hyperplasia (CAH), cystic fibrosis (CF), Charcot-Marie-Tooth disease (CMT), connexin 26 (CNX26), myotonic dystrophy (DM), Duchenne & Becker muscular dystrophies (DMD/BMD), familial adenomatous polyposis coli (FAP), fragile X syndrome (FRAX), Friedreich ataxia (FRDA), Huntington disease (HD), hereditary non-polyposis colon cancer (HNPCC), hereditary recurrent fevers (HRF) - Pilot, multiple endocrine neoplasia Type 2 (MEN2), mitochondrial myopathy (MM), monogenic diabetes (MonoDiab), phenylketonuria (PKU), porphyria (POR), Prader Willi & Angelman syndromes (PWAS), retinoblastoma (RB), spinocerebellar ataxias (SCA), spinal muscular atrophy (SMA), Von Hippel Landau syndrome (VHL), Wilson disease. Also a methods based DNA sequence and analysis scheme and cytogenetics are also offered.	3 samples are sent out per disease module, 1 X/year	Extracted DNA or RNA, lyophilized DNA [through EMQN]; amplified DNA products and sequencing primers	Analytical, post-analytical
Istituto Superiore di Sanita (ISS)	Cystic fibrosis, beta thalassemia, fragile X syndrome, adenomatous polyposis of colon	6 samples per disease	DNA from lymphoblastoid cells	Analytical and post-analytical
Reference Institute for Bioanalytics (DGKL)[42]	*Molecular Biology schemes:* FV-Leiden, prothrombin, MTHFR (C677T, A1298C), PAI-I 4g5g, FXIII V34L, GPIIIa, βFib g-455a, VKORC1 (g-1639a/c1173t), FXII c46t, FV H1299R, a1	*Molecular Biology schemes:* 2X/yr; *Sequencing-based scheme:* 2X/yr, DNA isolation: 2 X/yr	*Molecular Biology schemes:* Lyophilized DNA. *Sequencing-based scheme*: 2 lyophilized DNA samples, *DNA*	*Molecular Biology schemes*: analytical. *method-based schemes*: sequence result and interpretation, DNA isolation: determination of concentration of DNA, ratio 260/280, method of identification, defined genotypes

PT/EQA Provider	Analytes Covered	Molecular Genetic Challenges Per Year	Sample Types	Phases of Testing Covered
	PI, Apo E, Apo B100, ACE, CETP, TPMT, Cyp2C19 *1/*2/*17, Cyp2D6, Cyp2C8 (K399R), Cyp2C9 *2/*3, UGT1a1 (*28), DPD Exon 14 skipping, BCHE A/K, ALDO B (149/174/334), HFE (H63D, C282Y, S65C), LCT c-13910t, NOD2 (R702W, G908R, L1007fins C), M. Wilson ATP7B-C3207 A, FSAP (Marburg-I), ITGA2 Gplalla C807T K-Ras: Codon 12/13/61, *Method schemes*: DNA sequencing, DNA isolation + FV genotyping		*isolation*: 2 tubes whole blood	
CDC Newborn Screening Quality Assurance Program (NSQAP)[37]	Dried blood spot testing for cystic fibrosis mutations	5 dried blood spots 4X/yr	Blood from CF patient spotted on filter paper and dried	Pre-analytical, analytical, interpretation

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Table 3

Disease-Specific Proficiency Testing Performance (as assessed from select published literature)

Source	Disease	Participation	General Performance	Analytical Errors Identified	Interpretation Errors Identified	Lessons Learned
CAP[53]	Huntington disease	33 US and 23 international laboratories	Analytic sensitivity 99.5% over 6 surveys	Repeat length errors occurred in 2.6% of 1,060 allelic challenges. Most errors were minor and occurred in a small subset of the laboratories. Non US participants had higher error rate (17.5%).	3 interpretation errors were made in US laboratories over the 6 surveys.	Performance was very good. An analytical method with a high error rate was identified.
CAP[52]	Fragile X syndrome	Average of 90 laboratories over study period. US + international.	Analytic sensitivity for detection of: full mutations 99% (males), 96% (females); premutations 98%	Measurement of repeat length was more accurate with smaller samples.	3 interpretation errors were made in samples with repeat sizes 20–33 over 18 suveys. A number of laboratories incorrectly identified samples with 42 repeats as gray-zone or premutation.	Accuracy of sizing and interpretation of larger repeats improved over time.
EMQN[70]	Spino-cerebellar ataxia	Only 28.8% labs participated in all consecutive years	97.5% of reports correctly identified genotype as normal or pathogenic.	Large interlaboratory variation seen in allele sizing. Gross diagnostic errors occurred every year (false negatives, false positives, sample misidentification). Not all labs reported allele size.	Wide range of allele sizing but without incorrect diagnoses. Clerical errors observed.	**1** One third of labs with inaccurate sizing did not participate the following year, and for those that did, more than half continued to reported wrong sizes. Importance of participation in PT every year. **2** Allele sizing should always be reported: it is a measure of technical quality and may give clinically important information.
EMQN[57]	BRCA1&2		Error rate over three years: 5.8 to 3.7%	Missed mutations (53% of false results). Problems with incorrect nomenclature.	Misinterpretati on of observed sequence changes	Horizontal comparison between labs allows for a better understanding of the state of the field and for detection of systematic technical failures.
European Concerted Action on	Cystic fibrosis	135 European labs, 1 in Australia	35% of laboratories incorrectly genotyped at least one o the 12 CFTR alleles in study	Mistyping, misinterpretation of data, erroneous technical results.		Regular participation in PT schemes would be beneficial for labs. Lab personnel should participate in regular training sessions. Need consensus

Source	Disease	Participation	General Performance	Analytical Errors Identified	Interpretation Errors Identified	Lessons Learned
Cystic Fibrosis framework[54]						strategies for diagnostic testing. Centralized mutation analysis facilities would be useful for identified rare mutations.
UKNEQAS[44,51]	Several diseases		From 1998–2005, genotyping error rate: 15% to 0%; interpretation error rate: 5% to 0%	Technical errors and wrong nomenclature.	Incomplete reports, wrong clinical interpretations	**1** Any poor performance results in immediate correspondence between the organizer and the lab. **2** Scheme located test validity problems as well as clinical practice problems with wider implications for the genetic testing community.
ISS[71]	Fragile X syndrome	65% of all Italian labs performing testing participated. 15 labs participated in each of the five years.	Overall success rate: 76%.	Overall, 5% of samples tested were incorrectly genotyped and 1.5% of this was due to one sample, a male mosaic normal/ full mutation which was incorrectly genotyped nine times (three by the same lab). Some technical failures due to the sole of presecreening methods.	Reports showed considerable variation. Starting in 2004, a model for reports was issued	**1** Importance of reports for patient care. **2** Importance of secondary screening methods
ISS[72]	Cystic fibrosis, fragile X syndrome,		Overall genotyping performance: ~90%, Interpretive performance varied by disease	7% of labs incorrectly genotyped two mutations although their commercial kit detects those mutations. Many labs did not include clinically relevant cystic fibrosis mutations in their panel.	Written reports were inadequate for over 50% of labs. The most common error was lack of risk calculation when a mutation was not detected.	**1** Even commercial kits must be validated in the lab prior to use for clinical testing. **2** Labs need guidance on the kinds of mutations to include in their panels. **3** Complete reports are crucial for patient care. **4** Laboratory errors were disease-specific and not indicative of

Source	Disease	Participation	General Performance	Analytical Errors Identified	Interpretation Errors Identified	Lessons Learned
						overall laboratory analytical performance overall laboratory analytical performance overall laboratory analytical performance
ISS[73]	beta-thalassemia	11–18 labs over 5 years	98.9% of alleles correctly identified			Laboratories did a good job of reporting some aspects of the test, such as diagnostic sensitivity, however, many reports did not identify the mutations tested, indication for genetic counseling, or interpretation of results.
RCPA (Royal College of Pathologists of Australasia)[74]	HFE	37 labs currently	99.47% success over 10 surveys	Most errors due to sample handling, result transcription. A few errors caused by technical issues.	Incorrect results due to transcriptional errors	Performance was very good, but labs should continue to participate in large QAPs to maintain high testing quality

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Table 4

Methods-Based Proficiency Testing Schemes and Performance

Source	Technology Assessed	Labs Provided With:	PT protocol	Laboratory Performance
CAP[75] (ongoing PT scheme)	aCGH	Extracted DNA from cell lines derived from individuals with a constitutional abnormality	Laboratories must: **1** Analyze specimens and identify and interpret results for clinically significant copy number abnormalities. **2** Identify and characterize gains or losses and the cytogenetic location of any abnormalities detected using standard nomenclature	95.7% of responses were concordant for an abnormality involving a particular chromosomal location. Errors included designating an opposite change, reporting a clinically insignificant copy number change or failure to report a clinically significant abnormality.
CAP (ongoing PT scheme)	Post-analytical DNA sequencing	One CD-ROM containing DNA sequence electropherogram files	Laboratories must interpret and report DNA sequence variants for inherited disease using standard nomenclature	No published data available
EQUAL[65] (pilot)	DNA extraction and amplification	**1** Two pre-extracted DNA samples, **2** Whole blood samples, **3** Primer sets	Laboratories must: **1** Perform DNA extraction by routine procedures, **2** Estimate DNA quality and quantity of both pre-extracted and lab-extracted DNA, **3** Perform PCR with 100 ng of DNA from all samples, **4** Submit raw data from DNA quantification and post-PCR interpretation, **5** Send aliquot of DNA extracted from whole blood back to EQUAL	25% of labs performed poorly in quantification of at least 1 of the 2 pre-extracted samples. 27% of labs had questionable results for quality and/or quantity of blood sample extractions. High degree of variability seen with PCR performance of all samples.
EQUAL[66] (pilot)	Real-time PCR	**1** ABL gene primers, **2** 5′-FAM/3′-TAMRA-labeled probes, **3** Five ABL standard plasmids (10E2-10E5), **4** Three test cDNA samples, **5** Two cell samples	Laboratories must: **1** Construct a calibration curve; **2** Estimate cDNA copy numbers in 3 cDNA samples; **3** Perform RNA extraction, real-time PCR, and cDNA quantification of the 2 cell samples; **4** Provide Ct values for NTC, calibrators, and unknowns	For the cDNA samples, 80% of labs provided accurate values for all three. For cell samples, 36% of labs provided 95% CI limits that fell outside the range of standard dilutions. Wide variation in lab results were observed.

Source	Technology Assessed	Labs Provided With:	PT protocol	Laboratory Performance
			plus details of the testing platforms used. plus details of the testing platforms used.	
EQUAL[62] (pilot)	Sequencing	**1** Primer sets, **2** Two plasmid DNAs (one is a commercial cloning vector and one is a mixture of 2 different plasmids generated to provoke comments with respect to template quality), **3** A purified PCR product, **4** A finished sequencing reaction to be purified and analyzed	Laboratories must: **1** Perform sequencing and provide longest possible sequence without any mistakes (primer-walking not allowed) for DNA plasmids and purified PCR product, **2** Purify and analyze the finished sequencing reaction.	For the cloning vector plasmid, the mean sequence stretch (of the 2355-bp insert) was 537 bases and only 14 of 43 participants identified the insert correctly. For the mixture of two different plasmids, 21% of labs did not register any kind of difficulty, however some excellent specific comments were received. For the finished PCR product, 57% of labs made no effort at identification and only 10% of labs were awarded all possible points.
EMQN[63] (on-going EQA scheme)	Sequencing	**1** 450-bp PCR-amplified fragments of CFTR covering all main types of sequence changes, **2** A wild-type control, **3** Primers (with location in sequence), **4** Reference sequence, **5** Protein translation	Laboratories must: **1** Return form with genotyping results, **2** Return color copies of electropherograms and electronic copies of sequence data files.	59% scored the maximum score of 12.0 marks. 19 (5%) genotyping errors were made from 346 genotypes analyzed; 10 (53%) were false negative results and 9 (47%) were false-positive results. 59% of errors were made in naming mutations. Most laboratories produced data with acceptable diagnostic quality.
ISS[64] (pilot)	DNA extraction, PCR performance, interpretation	**1** Three primer pairs, **2** One reference DNA, **3** Three DNA calibrators **4** One blood sample	Laboratories must: **1** Extract DNA from blood sample and measure quality and quantity, **2** Measure quantity and quality of 3 standard DNAs, **3** Perform PCR, **4** Return PCR results, extracted DNA aliqout, and PCR product aliqouts	Variability was high for DNA quantification (ranged from 0.012 to 0.54 μg/μl with a CV of 82%). The 260/280 ratios ranged from 0.8 to 2.5 with a CV of 21%. A large variability was seen in the yield and quality of PCR performance. Fewer than 50% of labs reported satisfactory results.
HGSA-ASOC (ongoing scheme)	Microarray analysis	Extracted DNA from an individual tested for a constitutional abnormality	Assessment of laboratory processing and/or analytical skills, as well as interpretive comments.	No published data available

Source	Technology Assessed	Labs Provided With:	PT protocol	Laboratory Performance
HGSA-MGSA (ongoing scheme)	DNA sequencing and interpretation	**1** Amplified products **2** Sequencing primers	Laboratories must: **1** Perform Sanger sequencing using PCR products and primers provided, **2** Analyze and report results using standard nomenclature and provide biological interpretation.	No published data available
Birch et al.[76]	DNA extraction, PCR amplification	**1** Tubes containing high, medium and low concentrations of bacteria in buffer, **2** Control DNA in buffer, **3** PCR reagents (dNTPs, primers, Taq polymerase, reaction buffer) **4** 100-bp DNA ladder, gel loading dye)	Laboratories must: **1** Extract DNA from suspended bacteria, **2** PCR amplify extracted DNA according to protocol provided, **3** Analyze using agarose gel electrophoresis	Identified analytic issues such as inappropriate extraction or amplification procedures, PCR inhibition or contamination, poor labeling/or poor quality gel photography, failure to record results correctly
DGKL (ongoing scheme)	DNA sequencing and interpretation	Lyophilized DNA	DNA sequencing and corresponding diagnostic interpretation	No published data available
DGKL (ongoing scheme)	DNA isolation, quantitation (and factor V genotyping)	Whole blood	Laboratories must: **1** Isolate DNA, **2** Determine the concentration of DNA, **3** Report 260/280 ratio, **4** Identify the factor V genotype of the sample.	No published data available
ISS[77] (pilot)	TaqMan real-time PCR	**1** Standard cDNA solution obtained by in vitro transcription of a fragment of hTERT cloned into a plasmid vector, **2** Mix of primers, **3** Three unknown cDNA samples, **4** PCR conditions	Laboratories must: **1** Prepare dilutions of the provided standard, **2** Perform PCR and analyze results, **3** Report Ct values for each well, **4** Report concentrations of unknown samples	Only 12 of 42 labs gave results that were both concise and accurate for all samples tested. 17 labs reported inaccurate data for at least one result. Inaccuracy showed an inverse dose-dependent trend. 12 labs were unable to measure a sample with low concentration.